Crayfish females eavesdrop on fighting males before choosing the dominant mate.

Supplemental data: Crayfish females eavesdrop on fighting males before choosing the dominant mate

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Supplemental Experimental procedures

Collection and holding conditions

About 150 individuals (females and Form I –reproductive- males) were collected using baited traps from the Fucecchio wetland (Tuscany, Italy) in July, before the onset of reproduction. Once in the laboratory, for each individual we measured the cephalothorax length (from the tip of the rostrum to the posterior edge of the carapace) as an index of body size and the length and width of the claws (i.e. the propodi of the first pereopods) using a vernier caliper to the nearest 0.1 mm. Crayfish were individually marked on their carapace with a waterproof paint. Sexes were kept apart in tanks (80 x 60 x 60 cm) containing clay pots as refuges at a density of 15 m^{-2}. They were maintained in a natural light-dark cycle at room temperature (28 °C) and fed *ad libitum* with live *Calliphora* sp. larvae. Water was changed weekly.

Criteria for choosing crayfish for experiments

We selected sexually-responsive hard-shelled crayfish with all appendages and the rostrum intact. Males were defined as sexually responsive when, once placed in the presence of a female, they tried to turn the female over for copulation. The mean (± SE) cephalothorax length of the test individuals was 44.36 (± 0.53) mm for males and 44.77 (± 0.60) mm for females. Because in crayfish male dominance increases with body size and females prefer large males, pairs of male interactancts were matched for body (± 2 mm) and claw (± 1 mm) size. The selected individuals were kept isolated in opaque plastic aquaria (25 x 15 x 25 cm) for at least a week. In no case did the crayfish meet each other prior to the experiment, so that we can exclude any effect of previous social experience. All crayfish were used only once to avoid pseudo-replication.

Experimental design

The experiment was composed of two phases and two treatments. In the first phase (“fight phase”), one female was either allowed (bystander) or impeded (naïve) to watch and smell two males fighting. In the second phase (“choice phase”), both types of female were allowed to choose between the dominant and the subordinate male. After each

trial, male interactants and females were again tested for their responsiveness to a sexual partner. If a crayfish was no more responsive, the corresponding trial was excluded from the analysis. Similarly excluded were the trials where dominance (see below) was not clearly established (i.e. less than 60%). The analyzed replicates were 15 per treatment.

Apparatus

It consisted of two elliptical aquaria (length: 65 cm; width 40 cm; water level: 10 cm), one used in the fight phase ("fight arena") and the other in the choice phase ("choice arena"). A T-shaped opaque PVC wall divided each aquarium into three chambers, the first for the female and the remaining two, of the same size, for a male each (Figure S1). The experiment began with the removal of the wall after 10-min acclimatization.

In the "fight arena" the two males were free to move whereas the female was inserted into a 22 cm-high container (10 x 4 cm) in the female chamber. The container was transparent and finely drilled (3 mm holes, 4 cm^{-2}) if the female was bystander and opaque and without holes if the female was naïve. In both cases, the container precluded any physical contact between the interactants and the female. In the "choice arena", the two males were fastened with a string at the opposite ends, whereas the female was free to move. The position of the dominant and subordinate males in the arena was randomized. Between trials, the experimental apparatus was thoroughly washed with clean tap water.

Collection of data

The experiment was conducted between July 19 and August 1, 2007 during 0800-1400 hours. Fight and choice phases ran for 30 minutes each, during which time crayfish behavior was video-taped using a Samsung digital camera (VP-L800). A code number was given to each tape for subsequent blind reading. The tapes were examined in random order by an observer, extraneous to the experimental design and to the authors' expectations but experienced in the description of crayfish behavior. For the fight phase, the observer recorded the percentage of dominance for each individual, i.e. the number of fights won on the overall number of fights in percentage. Fights were deemed to begin when one opponent approached the other and to end when one of the two individuals (the looser) ran away, backed off or, tail flipped away from the other at a distance longer than one body length for at least 10 sec. For the choice phase, the observer recorded:

(1) The first male visited. A female was deemed to visit a male when she approached him within a distance of less than one body length. We excluded instances in which the female approached males by swimming or walking backwards.

(2) The total and mean duration of visits in seconds, as an estimate of the female persistence in her choice. The visit was deemed to end when the female retreated to a distance of more than one body length.

(3) The total number of pre-copulatory contacts (i.e. touches and pushes) between the female and the visited male.

(4) The time (in sec) spent by males with raised claws as an index of their dominance.

Only at the end of tape reading was the dominance computed and was the correspondence given between the chosen males and their status.

Statistical analyses

The total and mean duration of visits and the time spent by males with raised claws were analyzed using two-tailed Student's *t*-tests for paired data (statistic: *t*) and two-way ANOVAs (statistic: *F*). Frequency data (the first male visited and the number of contacts) were analyzed using *G* tests (statistic: *G*) and Wilcoxon tests (statistic: *Z*).

Supplemental Acknowledgements

The work was made possible by grants of the University of Florence to L.A. and F.G. and of the Czech Ministry of Education (No. MSM 600766809) and the Czech Science Foundation (No. GAAV IAA601870701) to M.B. We thank Carmen Trunfio for her help in tape reading. We are grateful to three anonymous referees and Prof. Geoffrey North for their helpful suggestions.

Figure S1. Scheme of the experimental apparatus. It consisted of two elliptical aquaria (length: 65 cm; width 40 cm; water level: 10 cm), one for the fight phase ("fight arena") and the other for the choice phase ("choice arena"). A T-shaped opaque PVC wall separated each aquarium in three chambers, the first for the female and the remaining two, of the same size, for a male each. The experiment began with the removal of the wall after 10-min acclimatization and the experiment ran for 30 min. In the "fight arena"(a) the two males were free to move whereas the female was inserted into a 22 cm-high container (10 x 4 cm) in the female chamber (broken line). The container was transparent and finely drilled (3 mm holes, 4 cm^{-2}) if the female was bystander and opaque and without drills if the female was naïve. In the "choice arena" (b), the two males were fastened with a string (⊣) at the opposite ends, whereas the female was free to move.